bio-protocol Preprint

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Primary hair cell culture

Louise Menendez Justin Ichida Neil Segil

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An abbreviated version of this protocol was published in eLIFE in Jun 2020

Generation of inner ear hair cells by direct lineage conversion of primary somatic cells

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Detailed protocol

Dissection steps of mouse Organ or Corti at P1

Prior to dissection prepare coated culture dishes with one of the following

- 10% Matrigel in hair cell media as a 20ul drop for droplet cultures
- 1:100 dilution of laminin in PBS for 96-well plate cultures

1. euthanize the P1 mice
2. remove the head and cut along the sagittal plane to have one ear in each half
3. carefully remove the soft brain tissue to expose the outline of the ear
4. remove the surrounding skull tissue to isolate the temporal bone cartilage
5. carefully dissect away the temporal bone cartilage to expose the cochlea and other inner ear structures
6. from the inner ear structures the central canal of the cochlea can be isolated by removing the modiolus and spiral ganglion neurons
    a. removal of the stria vascularis and Reisner’s membrane will better isolate the organ of Corti
        i. at this stage the prep will still contain the basilar membrane, LER, GER and Kolliker’s organ
7. transfer the isolated organs of Corti to an Eppendorf tube with 500ul of 0.25% trypsin
8. allow the organs of Corti to sit in the trypsin at 37C for 5 minutes
9. triturate the suspension for 30 sec and continue incubation for another 5 minutes at 37C
10. after incubation for a total of 10 minutes, triturate the sample for 2 minutes
11. add a small volume of FBS or media with FBS to the tube to quench the trypsin activity
12. centrifuge the tube at 600g for 5 minutes
13. remove the supernatant and resuspend the cell pellet in a fixed volume of Hair Cell Media (HCM) for quantification
14. quantify the cells (yields are ~100,000 cells per cochlea but can vary with dissection quality)
15. plate the cells at high density for optimal survival (2,500-3,000 cells/ul) in HCM with Rock Inhibitor (RI)
    a. plating density should be optimized for each experimental design
    b. for Matrigel droplet cultures, plate cells in a 30 ul drop and flood wells with hair cell media after 3-4 hours
    c. for 96-well laminin coated plates, plate cells in a 50 ul drop and flood wells with hair cell media after 3-4 hours
16. media change after 24 hours to remove RI and add fresh HCM

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Menendez, L. , Ichida, J. and Segil, N. (2022). Primary hair cell culture. Bio-protocol Preprint. bio-protocol.org/prep1954.
2. Menendez, L., Trecek, T., Gopalakrishnan, S., Tao, L., Markowitz, A. L., Yu, H. V., Wang, X., Llamas, J., Huang, C., Lee, J., Kalluri, R., Ichida, J. and Segil, N.(2020). Generation of inner ear hair cells by direct lineage conversion of primary somatic cells. eLIFE. DOI: 10.7554/eLife.55249

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